

Amendments to the Specification:

Please replace the paragraph on page 1, line 1, introduced in the preliminary amendment of January 14, 2004, with the following amended paragraph:

The present application is a divisional application of United States patent application number 09/967,301 filed September 28, 2001, now patented under United States patent number {number will be inserted once received}, which claims priority to patent application number 0109858.1 filed in Great Britain on April 23, 2001, the entire disclosures of which are hereby incorporated by reference.

Please replace the paragraph on page 16, lines 17-20, with the following amended paragraph:

The DNA construct may also be prepared by polymerase chain reaction (~~PCR~~) (PCRTM) using specific primers, for instance as described in US 4683202 or by Saiki et al (Science (1988), 239, 487-491). A recent review of ~~PCR~~-PCRTM (polymerase chain reaction) methods may be found in PCR Protocols, (1990), Academic Press, San Diego, California, USA.

Please replace the paragraph on page 31, lines 5-11, with the following amended paragraph:

The GFP gene used in the present study was contained within the plasmid pGFP (Chalfie et al., Science, (1994), 263, 802-805; GenBank accession number U17997) obtained from Clontech Laboratories Inc. (Palo Alto, Ca, USA). The gene was amplified by ~~PCR~~ PCRTM (polymerase chain reaction) using Pfu polymerase (Promega, Madison, WI, USA) according to recognised protocols (Saiki et al., Science, (1988), 239, 487-491). The sequences of primers used were:

GFP-1	5'-ggtacgggcccaccatgagtaaaggagaagaactttcac	SEQ ID NO: 5
GFP-2	5'-ggtacgggtaaccggtttgtatagttcatccatg	SEQ ID NO: 6
GFP-3	5'-ggtacgggcccaccatgggatccaaaggagaagaactttcac	SEQ ID NO: 7

Please replace the paragraph on page 31 line 13, through page 32, line 5, with the following amended paragraph:

Primer GFP-1 exhibits homology to the 5' region of the GFP gene and contains a partial Kozak site (Kozak, M, Cell, (1986), 44, 283) prior to the start codon for efficient initiation of translation in mammalian systems. Primer GFP-2 exhibits homology to the 3' region of the GFP gene and contains an additional *AgeI* restriction enzyme site immediately prior to the stop codon to facilitate cloning of proteins by fusion to the C-terminus of GFP. Primer GFP-3 is similar to primer GFP-1 exhibiting homology to the 5'

region of the GFP gene, but contains an additional restriction site (*Bam*HI) immediately after the initiation codon to facilitate cloning of proteins by fusion to the N-terminus of GFP. Amplified products resulting from ~~PCR~~PCRTM (polymerase chain reaction) reactions containing primers GFP-1 and GFP-2, and GFP-3 and GFP-2 were tailed with a single 3'-deoxyadenosine using Taq polymerase (Amersham Pharmacia Biotech, Amersham, UK) and ligated into the TA cloning vector pTARGET (Promega) according to manufacturer's instructions. The correct orientation relative to the CMV promoter and sequence of the insert was determined by automated DNA sequencing.

Please replace the paragraph on page 34, line 23 through page 35, line 15, with the following amended paragraph:

The gene for the mutant F64L-S175G-E222G-GFP (Example 2) was excised from pTARGET with *Bam*HI and *Sal*I and sub-cloned into the IPTG-inducible, GST-fusion vector pGEX-6P1 (Amersham Pharmacia Biotech). *E. coli* JM109 cells (Promega) containing an expression vector with the GST-GFP gene fusion were grown at 30°C to an OD₆₀₀=0.6 in 2x YT broth containing 100 µg/ml ampicillin. Protein expression was induced with IPTG (0.1 mM) and incubation continued for 16 hours. Cells were pelleted by centrifugation, resuspended in PBS and lysed by sonication (four 10 second bursts at 20 µm with intermittent cooling on ice). Cellular debris was removed by centrifugation and the lysate containing soluble GST-GFP fusion protein was purified using glutathione

sepharose columns (Amersham Pharmacia Biotech). Protein was then exchanged and eluted in PBS using a PD10 column (Amersham Pharmacia Biotech). The presence of a single band of correct molecular weight in the protein preparation was confirmed by SDS-PAGE using 4-12% ~~Bis-Tris NuPAGE gel electrophoresis (Invitrogen)~~ Bis-TRIS[®] HCL (hydroxymethyl) aminomethane hydrochloride buffered polyacrylamide gel sold under the trademark NuPAGE by Invitrogen. To assess protein concentration and purity, the protein preparation was subjected, in duplicate, to acid hydrolysis and filtration before amino acid analysis by ion exchange chromatography using a Pharmacia alpha plus series II analyser.

Please replace the paragraph on page 35, line 17 through page 36, line 2, with the following amended paragraph:

The extinction coefficient (Table 2) was determined on a UV/vis spectrometer (Unicam). Quantum yield (Table 2) was determined according to the method documented by Patterson et al (Biophysical Journal, (1997), 73, 2782-2790). Samples of equal optical density at respective absorbance maxima were prepared, and diluted, in 10mM ~~Tris-HCl~~ TRIS[®] HCL (hydroxymethyl) aminomethane hydrochloride pH 8 for the purified GFP preparation and a fluorescein reference standard (Molecular Probes). Fluorescence emission was measured in the region 490 – 600nm using a LS50B

luminescence spectrometer (Perkin Elmer) and results for the GFP preparation were compared directly to those for the fluorescein standard (QY=0.85).

Please replace the paragraph on page 37, lines 7-11, with the following amended paragraph:

The human NFκB P65 subunit gene (GenBank Accession number: M62399) was amplified using PCR PCRTM (polymerase chain reaction) according to recognised protocols (Saiki et al., Science, (1988), 239, 487-491). The sequences of primers used were:

NF κ B-1 5'-tttactcgagatggacgaactgttccccctca

SEQ ID NO: 18

NFκB-2 5'-tttgaagcttggagctgatctgactcagcagg

SEQ ID NO: 19